asserting that the feature "adding primer sequences" is allegedly lacking antecedent basis in the specification.

Claims 1-6 are rejected under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 1-7 of U.S. Patent No. 6,159,690 (hereinafter '690 patent) in view of Kikuchi et al. (*Gene*, Vol. 243, pp. 133-137, 2000).

At page 5 of the Official Action, the Examiner has rejected claim 1 under 35 U.S.C. §102(a) as allegedly being anticipated by Kikuchi et al. (*Supra*).

Finally, at page 6, the Examiner has rejected claims 2-6 under 35 U.S.C. §103(a) as allegedly unpatentable over Kikuchi et al. (*Supra*) and '690 patent.

The foregoing constitutes the entirety of the objections and rejections raised in the December 17, 2002 Official Action. In light of the Terminal Disclaimer under 37 C.F.R. §1.321, the Declaration of Professor Mathias Uhlen under 37 C.F.R. §1.132 and experimental evidence, (all of which is submitted herewith), and the following remarks, each of the above-noted rejections under the judicially created doctrine of obviousness-type double patenting and 35 U.S.C. §§112, second paragraph, 102(a), and 103(a) is respectfully traversed.

There is Sufficient Antecedent Basis for the Recitation of "Adding Primer Sequences" in Claim 2

The Examiner alleges the phrase "adding primer sequences" in step c) of claim 2 lacks antecedent basis. This allegation is respectfully traversed.

Applicants are entitled to present claims of varying scope. It is clear that the practice of the method of claim 1, wherein no primer sequences are added, will result in the generation of a population of variant polynucleotide sequences

due to the annealing the exonuclease digested single stranded fragments. The specification and claims as originally filed explicitly disclose that the addition of these primers is optional. The Examiner's attention is directed to page 6, lines 10-15 for support for this assertion. The Examiner is also directed to page 20, lines 11-16 of the specification, wherein it is specified that the methods of the present invention comprise the step of mixing the two pools of single-stranded DNA fragments in equimolar amounts, followed by two subsequent PCR reactions, wherein the **first** PCR reaction contains no primer. At page 27, lines 10-21 of the specification, it is further provided that during the second PCR reaction, a pair of forward and reverse PCR primers were added to the reaction mixture. This disclosure provides clear support for new claim 7, thus, "optionally adding primer sequences" in step c) of claim 2 and in new claim 7 merely refers to the addition of primers during a second, optional PCR reaction. It is, therefore, submitted that the feature of "adding primer sequences" is fully supported by the specification as filed and the rejection should be withdrawn.

Claims 1-6 are Patentable over U.S. Patent No. 6,159,690 in view of Kikuchi et al. in Light of the Terminal Disclaimer Submitted Herewith

In response to the Examiner's rejection of claims 1-6 under the judicially created doctrine of obviousness-type double patenting, submitted herewith a terminal disclaimer disclaiming the terminal part of the statutory term of any patent granted on the above-identified application beyond the expiration date of the full statutory term of US Patent No. 6,159,690. In view of this terminal disclaimer, withdrawal of rejection to claims 1-6 is respectfully requested.

Claim 1 is not Anticipated by Kikuchi et al.

Applicants respectfully disagree with the Examiner's assertion that Kikuchi et al. teach and every element of claim 1. Specifically, the present invention relates to a method for generating a polynucleotide sequence(s) from single-stranded parent polynucleotide sequences, which method comprises the digestion of single-stranded parent polynucleotide sequences with an exonuclease, allowing the resultant single-stranded DNA fragments to anneal to each other, and the amplification of the annealed DNA fragments. Contrary to the Examiner's assertion that Kikuchi et al. teach "digesting each single stranded polynucleotide sequences with an exonuclease ..." (see page 5 of the Official Action), Kikuchi et al. teach the use of DNase I, an endonuclease, for the generation of single stranded polynucleotide sequences.

Endonucleases, such as DNase I, are enzymes that catalyze cleavage of nucleic acids internally, while exonuclease, such as BAL31, catalyze the cleavage of nucleotides from the ends of the nucleic acid molecule. As set forth in the Encyclopedia of Molecular Biology, Kendrew et al. 1994, an endonuclease is defined as an enzyme that cleaves phosphodiester bonds within a nucleic acid chain while an exonuclease is defined as a nuclease that degrades nucleic acids by cleaving successive nucleotide residues from the ends of the strands. In light of the above, it cannot be maintained that Kikuchi et al. anticipate the invention as claimed in claim 1. Accordingly, the §102(a) rejection should be withdrawn.

Claims 2-6 are Patentable over Kikuchi et al. in view of U.S. Patent No. 6,159,690

The Examiner's rejection to claims 2-6 as allegedly unpatentable over Kikuchi et al. in view of '690 patent is respectfully traversed.

In view of the terminal disclaimer submitted herewith Applicants respectfully submit that the rejection of claims 2-6 based on the combination of Kikuchi et al. and the '690 patent application has been obviated.

Claims 2-6 of the present application are dependent from claim 1, wherein the exonuclease is BAL31 or the parent polynucleotide sequences have been subject of mutagenesis.

As discussed above, Kikuchi et al. fails to teach the use of an exonuclease in digesting parent single-stranded polynucleotide sequences. Claims 2-6 of the present application, therefore, are not anticipated by Kikuchi et al.

As futher evidence of non-obviousness, a Declaration by Professor Mathias Uhlen and supporting experimental data is submitted herewith. Applicants respectfully submit that the use of an exonuclease to digest the parent DNA provides an unexpected advantage, namely the improved control of fragment size, which in turn permits greater control of the variant polynucleotides produced by the methods of the present invention (see Appendix 2 showing the Experimental evidence in support of Professor Uhlen's declaration). It is submitted that the endonuclease used by Kikuchi et al, DNase I in particular, would cleave nucleic acids to fragments with different sizes depending on the presence of Mg or Mn ions. Mg ions restrict the fragment size to 50 bp, while the Mn ions will give fragment size less than 50 bp. Therefore, in order to have all possible sizes for recombination the parent polynucleotide sequences need to be treated at least twice with DNase I in the presence of either of the two different ions, followed by removal of these very same ions. In the present application, an exonuclease, such as BAL31, is used for the cleavage of parent polynucleotide sequences, wherein a set of progressively shortened nucleic acid fragments can be easily obtained by controlling the reaction time.

In light of all the foregoing, Applicants respectfully request that this application be passed to issue.

Conclusion

It is respectfully urged that the rejections set forth in the December 17, 2002 Official Action be withdrawn. In the event the Examiner is not persuaded as to the allowability of any claim, and it appears that any outstanding issues may be resolved through a telephone interview, the Examiner is requested to telephone the undersigned attorney at the phone number given below.

Respectfully submitted,

DANN, DORFMAN, HERRELL AND SKILLMAN
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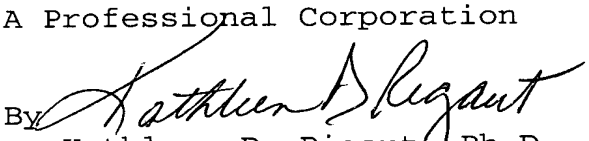
By 
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Marked up Draft of Amended Claims

2. (Twice Amended) [A] The method as claimed in claim 1 wherein step (c) optionally comprises adding primer sequences that anneal to the 3' and 5' ends of at least one of the parent polynucleotides under annealing conditions.